PATENT SPECIFICATION (11) 1 472 154

1 472 154

(21) Application No. 19840/75 (22) Filed 12 May 1975

(31) Convention Application No. 2423390 (32) Filed 14 May 1974 in (19)

(33) Federal Republic of Germany (DT)

(44) Complete Specification published 4 May 1977

(51) INT CL² C07D 207/28 C07C 103/52//125/06

(52) Index at acceptance

C2C 1341 20Y 215 220 226 227 22Y 247 250 251 25Y 280 282 29X 29Y 304 305 30Y 321 32Y 340 342 34Y 351 352 364 366 367 368 36Y 45X 45Y 571 57Y 584 620 625 627 628 62X 63X 658 65X 699 70Y 790 KA KB KJ KM KZ LU

C3H A2

(54) PYROGLUTAMYL COMPOUNDS AND PROCESS FOR THEIR MANUFACTURE

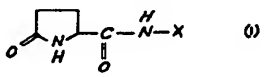
(71) We, HOECHST AKTIENGESELLSCHAFT, a body corporate organised according to the laws of the Federal Republic of Germany, of 6230 Frankfurt (Main) 80, Postfach 80 03 20, Federal Republic of Germany, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement: —

The present invention relates to pyroglutamyl compounds and to a process for their manufacture.

It is known that the tripeptide amide L-pyroglutamyl-L-histidyl-L-prolinamide (TRH), which is thyrotropic hormone releasing factor, has an antidepressive effect. However, in its use antidepressive agent, this peptide has the drawback of stimulating the secretion of thyrotropic hormone.

The present invention is based on the observation that simple pyroglutamyl amides show a high antidepressive effect in the dopa potentiating test but do not stimulate thyrotropic hormone secretion.

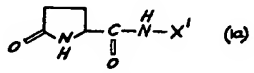
The present invention provides a pyroglutamyl compound of the formula I



(I)

wherein X represents a straight or branched chain alkyl radical having from 2 to 6 carbon atoms which is substituted in the α-position by a carbamoyl group or in the ω-position by a carbamoyl or carboxyl group.

This invention also provides a process for the manufacture of a compound of the general formula Ia



(Ia)

in which X′ represents a straight or branched chain alkyl radical having from 4 to 6 carbon atoms or a straight or branched chain alkyl radical having from 2 to 6 carbon atoms which is substituted in the α-position by a carbamoyl group or in the ω-position by a carbamoyl or carboxyl group, wherein either

a) a pyroglutamic acid, which may be substituted on the nitrogen atom by a benzyloxycarbonyl radical, and which is in the form of an active ester, a mixed anhydride, or a carbodiimide, is reacted with a primary amine of the general formula II

$$H_2N—X' \qquad (II)$$

wherein X′ is as defined above and any carboxyl groups present may be protected by suitable protective groups used in peptide chemistry, these protective groups being split off during or after formation of the compound of formula I, or

b) a glutamine derivative of the formula III

(III)

wherein X′ is as defined above, is cyclized to a compound of the formula I of the invention by boiling in trifluoroacetic acid or by allowing it to stand for a prolonged period of time in an aqueous solvent.

In method a), an active ester of the pyroglutamic acid is reacted with a primary amine. Suitable active esters are 4-nitrophenyl, 2,4,5-trichlorophenyl and pentachlorophenyl esters. Suitable esters for the protection of the carboxyl groups by which the amines can be substituted are tertiary butyl esters, which can be split off by an acid, benzyl and 4-nitrobenzyl esters, which can be split off by an alkaline agent or by catalytic hydrogenation, and alkyl esters, which can be hydrolysed by an alkaline agent, for example, methyl and ethyl esters. The reaction is preferably carried out in a strongly polar solvent, for example, dimethylformamide, N,N-dimethylacetamide or dimethyl sulphoxide, and it can be accelerated by the addition of 1-hydroxybenzotriazole or a similar compound (cf. German Patent Specification No. 2,202,613). A pyroglutamyl derivative that still contains carboxyl protecting groups is then converted into the compound of the invention by splitting off these groups in usual manner, for example, tertiary butyl esters are split off by acids, alkyl or aralkyl esters by bases, and esters of the benzyl type by catalytic hydrogenation.

In method b), there is advantageously used a glutaminyl derivative which can easily be cyclisized to the corresponding pyroglutamyl compound of the invention after splitting off the protective groups by boiling in trifluoroacetic acid, or by allowing the product to stand for a prolonged period of time in an aqueous solvent.

The glutaminyl derivatives themselves can be manufactured according to the usual methods of peptide chemistry, by reacting a glutamine derivative having a protected amino group and optionally also a protected carbamoyl group with the corresponding amino component, any carboxyl groups present in the amino components being blocked by the tertiary butyl radical. For example, the aminolysis of a benzyloxycarbonyl or tertiary butoxycarbonylglutamine *p*-nitrophenyl ester yields the corresponding protected glutaminyl derivative. By adding a suitable N-hydroxy compound, for example, 1-hydroxybenzotriazole or 1-hydroxypyrid-2-one the aminolysis can be accelerated considerably. Protective groups on the carbamoyl group of glutamine, for example, the 4,4′-dimethoxybenzhydryl radical, render the glutaminyl derivatives water-insoluble, and can be isolated easily.

The process of the invention enables, for example, the following compounds to be manufactured: L-pyroglutamyl-L-alanine amide, L-pyroglutamyl-L-leucine amide, L-pyroglutamyl-L-norleucine amide, L-pyroglutamyl-L-valine amide, L-pyroglutamyl-L-isoleucine amide, L-pyroglutamyl-β-alanine, L-pyroglutamyl-β-alanine amide, L-pyroglutamyl-4-aminobutyric acid, L-pyroglutamyl-4-aminobutyramide, L-pyroglutamyl-γ-aminovaleric acid, L-pyroglutamyl-γ-aminovaleramide, L-pyroglutamyl-6-aminohexanoic acid, L-pyroglutamyl-6-aminohexanamide.

In the dopa potentiating test in mice the compounds of formula I show an effect similar to that of thyrotropic hormone releasing factor itself. For this reason, the compounds of the invention may be used for the treatment of psychotic diseases, especially depressive illnesses.

The following is a list of values obtained in the dopa potentiating test for a number of compounds of the invention. The numbers are a combination of values obtained after 15 to 30 minutes after administration:

	Dopa potentiating test Superexcitation in % depending on the dose (mg/kg)			
	0.5	1	2	5
TRH	130	186	267	239
L-pyroglutamyl-L-analine amide	178	132	137	151
L-pyroglutamyl-β-alanine	192	167	162	204
L-pyroglutamyl-4-aminobutyric acid	140	125	255	245
L-pyroglutamyl-4-aminobutyramide acid	183	233	160	198
control	100	100	100	100

The following Examples illustrate the invention:

EXAMPLE 1

L-Pyroglutamyl-L-alanine amide

3.75 g (10 mmols) of L-pyroglutamic acid pentachlorophenyl ester were stirred in 40 ml of dimethylformamide with 1.5 g (12 mmols) of L-alanine amide/hydrochloride and 1.54 ml (12 mmols) of N-ethylmorpholine for 4 hours, the solvent was distilled of under vacuum, the residue was dissolved in methanol and the solution was stirred successively with a strongly basic and with a strongly acid ion exchange resin, the exchange resin was filtered off and the solution concentrated. The residue was triturated with ethyl acetate, dissolved in a little ethanol and precipitated with petroleum ether in the form of a resin that solidified under petroleum ether.

Yield: 1.2 g, chromatographically uniform without sharp melting point.

Calculated:	N 21.0
Found:	21.2

EXAMPLE 2

L-pyroglutamyl-β-alanine

6.75 g (0.05 mol) of 1-hydroxybenztriazole and 6.5 g of L-pyroglutamic acid in the form of a fine powder were added to a solution of 9.05 g (0.05 mol) of β-alanine tert. butyl ester hydrochloride and 6.32 ml (0.05 mol) of N-ethylmorpholine in 75 ml of dimethylformamide. The solution was cooled to −5°C and 11.3 g (0.054 mol) of dicyclohexylcarbodiimide dissolved in 25 ml of dimethylformamide were added. The solution was again stirred at 0°C for 6 hours and it was left for another 16 hours at +4°C. The precipitated N,N'-dicyclohexylurea was suction-filtered off, the filtrate was concentrated under vacuum at room temperature until it became syrupy. The residue was triturated twice with 180 ml of absolute diethyl ether, dissolved in 80% methanol and filtered through 200 ml of Serdolit-Blau (OH^--form). The ion exchange resin was washed with 350 ml of 80% v/v methanol, the eluate and washing solutions were combined and evaporated at room temperature, the remaining oil was dissolved in 30 ml of 90% trifluoroacetic acid and the solution was stirred for 1 hour at room temperature. The trifluoroacetic acid was evaporated under vacuum and the residue was triturated with absolute diethyl ether.

The crude product so obtained was recrystallized from ethanol/diethyl ether.

Yield: 6.4 g, m.p.: 199°C $[\alpha]_D^{22} = -8.3°$ (c=1, methanol).

EXAMPLE 3

L-pyroglutamyl-4-aminobutyric acid

a) N-Benzyloxycarbonyl-4-aminobutyric acid tert. butyl ester

600 ml of liquefied isobutylene and 6 ml of conc. H_2SO_4 were added to a solution of 172 g of N-benzyloxycarbonyl-4-aminobutyric acid in 600 ml of methylene chloride, the solution was shaken for 3 days at room temperature in an autoclave, and then the isobutylene was distilled off. The methylene chloride solution was washed twice with 10% w/v sodium carbonate solution and once with water, dried over sodium sulfate and concentrated under vacuum. The residue was dissolved in diethyl ether and chromatographed over 620 g of basic Al_2O_3. Elution with diethyl ether followed.

Yield: 161.9 g (oil).

b) 4-Aminobutyric acid tert.-butyl ester hydrochloride

A solution of 161.5 g of N-benzyloxycarbonyl-4-aminobutyric acid *tert.*-butyl ester in 500 ml of methanol was catalytically hydrogenated after the addition of Pd-catalyst and methanolic HCl at pH 4.5 (autotitrator). The reaction being finished (no further consumption of methanolic HCl) the catalyst was suction-filtered off and the filtrate was concentrated. The residue was dissolved in diethyl ether and cooled. After some time, a precipitate appeared which was suction-filtered.

Yield: 78.2 g, m.p.: 82—84°C.

c) L-Pyroglutamyl-4-aminobutyric acid tert.-butyl ester

1.3 ml of N-ethylmorpholine and 3.08 g of L-pyroglutamic acid 2,4,5-trichlorophenyl ester were added to a solution of 1.95 g (10 mmols) of 4-aminobutyric acid tert.-butyl ester hydrochloride in 20 ml of dimethylformamide. The solution was left overnight at room temperature and concentrated. The residue was dissolved in a mixture of ethyl acetate and water, the ethyl acetate was separated and shaken with sodium bicarbonate solution, $KHSO_4$-solution and NaCl-solution, dried with Na_2SO_4 and concentrated. The residue was triturated with petroleum ether.

Yield: 2.1 g, m.p.: 68°C.

d) L-Pyroglutamyl-4-aminobutyric acid

2 g of L-pyroglutamyl-4-aminobutyric acid *tert.*-butyl ester were dissolved in 20 ml of trifluoroacetic acid with warming, the solution was left at room temperature for 30 minutes, the trifluoroacetic acid was removed under vacuum and the residue was triturated with diethyl ether and suction-filtered. The product was dissolved in water, insoluble substances were removed by filtration over active charcoal and the solution was lyophilized.

Yield: 760 mg, m.p.: 117—120°, $[\alpha]_D^{20} = -11.3°$ (c=1, methanol.

$C_9H_{14}N_2O_4$ (214.2) Calculated: C 50.42 H 6.58 N 13.08
Found: C 50.6 H 6.5 N 13.2

EXAMPLE 4

L-Pyroglutamyl-4-aminobutyric acid

a) N^{α}-Benzyloxycarbonyl-N^{γ}-4,4'-dimethoxybenzhydryl-L-glutaminyl-4-aminobutyric acid tert.-butyl ester

2.53 g (5 mmols) of N^{α}-benzyloxycarbonyl-N^{γ}-4,4'-dimethoxybenzhydryl-L-glutamine, 975 mg of 4-aminobutyric acid *tert.*-butyl ester hydrochloride and 675 mg of 1-hydroxybenzotriazole were dissolved in 10 ml of dimethyl formamide. 0.65 ml of N-ethylmorpholine and, at 0°C, a solution of 1.1 g of dicyclohexylcarbodiimide in dimethylformamide were added. The solution was stirred for 1 hour at 0°C and left overnight at room temperature. The precipitate was suction-filtered off and the filtrate was concentrated. The residue was triturated with sodium bicarbonate solution and water, suction-filtered and dried over P_2O_5. The solid was boiled with acetone, cooled to 0°C, suction-filtered and washed with acetone and petroleum ether.

Yield: 3.45 g, m.p.: 178°C.

b) L-Pyroglutamyl-4-aminobutyric acid

2.5 g of N^{α}-benzyloxycarbonyl-N^{γ}-4,4'-dimethoxybenzhydryl-L-glutaminyl-4-aminobutyric acid *tert.*-butylester were boiled under reflux for 100 minutes with 2 ml of anisole in 20 ml of trifluoroacetic acid. Concentration followed and the residue was dissolved in a mixture of water and diethyl ether. The aqueous phase was clarified with active charcoal and lyophilized. The oily residue was recrystallized from methanol/diethyl ether.

Yield: 410 mg (about 50%). m.p.: 123—124°. Chromatographically identical with the material obtained according to Example 3d).

EXAMPLE 5

L-Pyroglutamyl-4-aminobutyramide

a) 4-Benzyloxycarbonylaminobutyramide

4.2 ml of triethylamine were added to a solution of 7.12 g (30 mmols) of N-benzyloxycarbonyl-4-aminobutyric acid in 50 ml of tetrahydrofuran. The solution was cooled to −10°C and 2.9 ml of ethyl chloroformate dissolved in 10 ml of absolute tetrahydrofuran were added dropwise. The solution was stirred for 10 minutes at −10°C, then 3 ml of liquid NH_3 were added and the solution was stirred for 1 hour at room temperature and the precipitate was suction-filtered off. The filtrate was concentrated and the residue was dissolved in a mixture of ethyl acetate and water. The ethyl acetate phase was washed with sodium bicarbonate solution and water, dried with sodium sulfate and concentrated. The residue was triturated with petroleum ether.

Yield: 5.4 g (76%); m.p.: 131—132°C.

b) 4-Aminobutyramide hydrochloride

4 g (16.9 mmols) of 4-benzyloxycarbonylaminobutyramide were catalytically hydrogenated in methanol in a manner analogous to Example 3b. The product crystallized after addition of diethyl ether.

Yield: 1.81 g (78%), m.p.: 137—138°C.

c) L-Pyroglutamyl-4-aminobutyramide

3.9 g (12.65 mmols) of L-pyroglutamic acid 2,4,5-trichlorophenyl ester were added to a solution of 1.76 g (12.9 mmols) of 4-aminobutyramide hydrochloride and 1.72 g (12.75 mmols) of 1-hydroxybenzotriazole and 1.65 ml of N-ethylmorpholine in 40 ml of dimethylformamide. The solution was left for 1 hour at room temperature, concentrated and triturated with diethyl ether. The substance was suction-filtered and chromatographed on Serdolit Blau using methanol/water (1:1 v/v) as eluant. The

eluate was concentrated and the residue was triturated with diethyl ether and suction-filtered.

Yield: 1.6 g (58%). For further purification, the product can be recrystallized from ethanol. M.p.: 159—160°C, $[\alpha]_D^{20} = -1.9°$ (c=1, methanol).

EXAMPLE 6

L-Pyroglutamyl-4-aminovaleric acid

a) L-Pyroglutamyl-4-aminovaleric acid ethyl ester

1.6 g (0.012 mol) of 1-hydroxybenzotriazole and 7.8 g (0.025 mol) of L-pyroglutamic acid 2,4,5-trichlorophenyl ester were added to a solution of 4.25 g (0.025 mol) of 4-aminovaleric acid ethyl ester hydrochloride and 3.2 ml of N-ethylmorpholine in 15 ml of dimethylformamide at 0°C. The solution was stirred at 0°C for 2 hours and then left for 16 hours at +4°C. It was evaporated under vacuum and the crude product was purified by filtration through an acid and a basic ion exchanger resin. The product was recrystallized from ethanol/diethyl ether.

Yield: 2.34 g, melting point: 74.5°C $[\alpha]_D^{22°} = +20.8°$ (c=1, dimethylformamide).

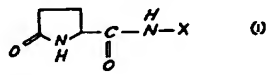
b) L-Pyroglutamyl-4-aminovaleric acid

1.13 g (5 mmols) of L-pyroglutamyl-4-aminovaleric acid ethyl ester were added to a solution of 800 mg (5 mmols) of barium hydroxide octahydrate in 35 ml of water, and the solution was stirred for 2.5 hours at room temperature. 2.5 ml (5 mmols) of 2N H_2SO_4 were added, the solution was filtered through a clarification layer of kieselguhr to eliminate the precipitated barium sulphate. The peptide was obtained in 80% yield as a colourless amorphous solid by lyophilization from the clear filtrate.

$[\alpha]_D^{22} = +7.3°$ (c=1, methanol).

WHAT WE CLAIM IS:—

1. A pyroglutamyl compound of general formula I

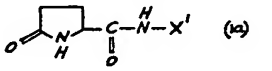


(I)

in which X represents a straight or branched chain alkyl radical having from 2 to 6 carbon atoms which is substituted in the α-position by a carbamoyl group or in the ω-position by a carbamoyl or carboxyl group.

2. A compound as claimed in claim 1 and which is described in any one of the Examples herein.

3. A process for the manufacture of a compound of the general formula Ia



(Ia)

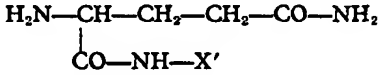
in which X' represents a straight or branched chain alkyl radical having from 4 to 6 carbon atoms or a straight or branched chain alkyl radical having from 2 to 6 carbon atoms which is substituted in the α-position by a carbamoyl group or in the ω-position by a carbamoyl or carboxyl group, which comprises

a) reacting a pyroglutamic acid which may be substituted at the nitrogen atom by a benzylaxycarbonyl radical and which is in the form of an active ester, a mixed anhydride, or a carbodiimide, with a primary amine of the formula II

$$H_2N—X'$$

in which X' is as defined above and in which any carboxyl groups present may be protected by suitable protective groups used in peptide chemistry, these protective groups being split off during or after formation of the compound of the formula I, or

b) cyclizing a glutamine derivative of the formula III



in which X' is as defined above to the corresponding compound of the general

formula I by boiling in trifluoroacetic acid or by allowing it to stand for a prolonged period of time in an aqueous solvent.

4. A process as claimed in claim 3, carried out substantially as described in any one of the Examples herein.

5. A compound of the general formula Ia as defined in claim 3, whenever produced by a process as claimed in claim 3 or claim 4.

ABEL & IMRAY,
Chartered Patent Agents,
Northumberland House,
303—306 High Holborn,
London, WC1V 7LH.

Printed for Her Majesty's Stationery Office, by the Courier Press, Leamington Spa, 1977
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.